EPA Reviewer: Nancy McCarroll **Signature:** _____
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** _____
EPA Secondary Reviewer: Christopher Schlosser, MFS **Signature:** _____
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** _____

TXR#: 0056765

DATA EVALUATION RECORD[1]

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in rat; OPPTS 870.5395 [' 84-2]; OECD 474.

PC CODE: 016331

DP BARCODE: DP410187

TEST MATERIAL (PURITY): Momfluorothrin (95.7% a.i.; Lot No. 9CM0109G)

SYNONYMS: S-1563

CITATION: Kitamoto, S. (2010). Micronucleus test on S-1563 in rats. Sumitomo Chemical Co., Ltd., Japan. Report No. RWT-0007, June 15, 2010. MRID 49020032. Unpublished

SPONSOR: Sumitomo Chemical Co., Ltd., Japan

EXECUTIVE SUMMARY: In a bone marrow micronucleus assay in rats (MRID 49020032), groups of 5 male and female Sprague-Dawley Crl:CD rats/sex/dose were treated orally by gavage with S-1563 (95.7% a.i.; Lot No. 9CM0109G) prepared as suspensions in corn oil at 0, 150, 300, or 600 mg/kg bw (♂) or 0, 50, 100, or 200 mg/kg bw (♀). Doses used in the main assay were based on the findings of a preliminary range-finding study; results showed death and other clinical signs (i.e., tremors and soft stools at 600 mg/kg in males and similar signs in females at ≥200 mg/kg). Bone marrow cells were harvested at 24 and 48 hours post-treatment.

The incidence of micronucleated cells in 2000 polychromatic erythrocytes (PCEs) was scored for each animal. Cytotoxicity was assessed by determining the ratio of PCEs polychromatic erythrocytes to whole erythrocytes (NCEs). Cyclophosphamide (CP) at 60 mg/kg served as the positive control substance.

In the main assay, tremors and soft stools were observed in males treated with ≥ 300 mg/kg; soft stools were also noted at 150 mg/kg. In the females, deaths and tremors were reported at 200 mg/kg. While there was no effect on the PCE: NCE ratio in the males, a significant decrease in the ratio was recorded for the females at 200 mg/kg after the 48-hour harvest. Nevertheless, no significant increase in incidence of micronuclei was seen in either sex at any dose or at any sampling time. The positive control (CP) induced an elevated and statistically significant increase in micronuclei at 24 hours.

1 Disclaimer: The attached Data Evaluation Record is a modified version of the Tier II Summary provided by Sumitomo Chemical Co. Ltd. Portions of this document may have been altered by the EPA reviewer.

Based on these findings, **there was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow of male or female rats treated with S-1563 at either sacrifice time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: S-1563
Description: Not stated
Lot/Batch: Lot No. 9CM0109G
Purity: 95.7%
CAS#: 609346-29-4
Stability: Stable for duration of study (analytically determined: 95.6% on March 2, 2010)
Solvent: Corn oil

2. Control materials:
Negative: Corn oil
Positive: Cyclophosphamide (CP, 60 mg/kg bw)

3. Test animals:
Species: Rat
Strain: Crl:CD (SD)
Age: 8 weeks at the time of dosing
Weight at dosing:

Range-finding (males)	254.2 – 297.2 g
Range finding (females)	178.9 – 206.7 g
Micronucleus test (males)	285.9 – 319.8 g
Micronucleus test (females)	191.2 – 236.1 g

Source: Charles River Japan, Inc., Hino Breeding Canter, Shiga, Japan
Number of animals per dose:

Dose-finding	5 animals per sex per dose
Main assay	5 animals per sex per dose and sampling time (in the high dose group a satellite group of 5 animals was set up to replace any that might die in the main group)
Controls	10 animals for the vehicle control group (corn oil) and 5 animals for the positive control group (CP)

Animal husbandry: Up to 3/cage in plastic cages with shavings
Environmental conditions

Temperature	22.3 – 23.7ºC
Humidity	38.1 – 69.5%
Air change	At least 10 air changes per hour
Photoperiod	12 hour light / dark cycle

5. Dose Levels

(a) Range finding (toxicity) test	Males: 150, 300 and 600 mg/kg bw (single doses)
	Females: 100, 200 and 400 mg/kg bw (single doses)
(b) Micronucleus assay:	Males: 150, 300 and 600 mg/kg bw (single doses)
	Females: 50, 100 and 200 mg/kg bw (single doses)

B. TEST PERFORMANCE

1. In life dates:

16 November 2009 to 9 February 2010

2. Treatment and sampling times:

a. Test compound and vehicle

Dosing:	[x]	once	[ ]	twice (24 hrs apart)			[ ]	Other)			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[x]	48 hr	[ ]	72 hr	
Other:											

b. Positive control:

Dosing:	[x]	once	[ ]	twice (24 hrs apart)			[ ]	Other			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[ ]	48 hr	[ ]	72 hr	
Other:											

3. Tissues and cells examined:

Were erythrocytes from bone marrow examined?:	Yes
No. of polychromatic erythrocytes (PCEs) examined per animal:	2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	One thousand total erythrocytes per animal were evaluated for bone marrow toxicity.
Other	

4. Details of slide preparation: Rats were killed by carbon dioxide asphyxiation. Marrow from the femurs of each animal was washed out with fetal bovine serum (FBS). The cell suspensions were centrifuged. The pellet of marrow cells was suspended in residual serum and a small drop was placed onto a microscope slide. Slides were fixed with methanol and coded. Prior to observation, acridine orange (40 µg/mL) was place onto the slides and slides were examined with a fluorescence microscope.

5. Statistics

Statistical analysis for the incidence of micronucleated PCE (MPCEs) was performed using the method of Kastenbaum-Bowman. The levels of significance were 5% and 1%.
The t-test was used for analysis of the ratio of PCEs and the animal body weight changes. The Student's t-test was used when significance was not detected at the level of 5% in the F-test and the Welch's t-test was used when significance was detected in the F-test. The levels of significance were 5% and 1% (two tailed).

6. Evaluation criteria

Criteria for acceptability of the assay:
The mean of the frequency of MPCEs in the negative control groups should be within the provided historical control range of the performing laboratory, and the frequency of MPCEs in the positive control group should be markedly increased with a statistically significant difference from the negative control group.

Positive response:

The test compound was judged to induce micronuclei if the following criteria were met:

- There was a statistically significant increase in the incidence of MPCEs in treated groups when compared to the concurrent vehicle control, and
- The increase was dose-related or reproducible.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test chemical was homogeneous and stable at 2 and 200 mg/mL in corn oil suspension for 6 hours at room temperature after storage in brown vial in refrigerator (1-15˚C) for 15 days. Thus, test material stability and homogeneity were confirmed analytically in corn oil.

B. RANGE FINDING (TOXICITY) TEST

Prior to the micronucleus test, a range finding toxicity test was conducted. S-1563 was administered at single oral doses of 0, 150, 300, and 600 mg/kg to groups of five male rats/dose and at 0, 100, 200, and 400 mg/kg to groups of five female rats/dose; clinical signs were observed for 2 days. Results indicated that one out of five male rats at 600 mg/kg was found dead within 1 day of S-1563 administration. In the female rats, one out of five at 200 mg/kg and four out of five at 400 mg/kg were found dead within 1 day of test material administration. Other signs of clinical toxicity in the males included tremors at 600 mg/kg and soft stool at ≥150 mg/kg. In female rats, clonic convulsions and soft stool were observed at 400 mg/kg; tremors were seen at ≥200 mg/kg. Based on these results, 600 mg/kg and 200 mg/kg, which slightly exceeded the maximum tolerated dose (MTD), were selected at the starting level for male and female rats in the micronucleus assay, respectively.

C. Micronucleus assay

1. Clinical signs:

In males clinical signs included: tremor observed 4 hours after administration at 300 and 600 mg/kg, and soft stool observed one day after administration at 150, 300 and 600 mg/kg. In females clinical signs included: death observed in two out of 20 animals at 200 mg/kg, tremor observed at 200 mg/kg 4 hours after administration. One female rat died within 24 hours at 200 mg/kg, so one rat from the satellite group was used for slide preparation. No deaths were observed in the 48 hour group at 200 mg/kg. Lethality and severe clinical signs at 600 mg/kg and 200 mg/kg in males and females in the dose finding and main assay indicate systemic absorption.

2. Bone marrow analysis:

Results of the micronucleus assay are summarized in Table 1 (males) and Table 2 (females). As shown, the incidence of MPCEs in the vehicle control group of both sexes slightly exceeded the historical control range of the performing laboratory (0.10 – 0.17% MPCE (N=5), ♂; 0.19 % MPCE (N=1),♀). However, the study author indicated that the incidences were within the range reported in the literature and, thus, were considered to be within the expected variation range for a "normal biological response". There were no significant increases in incidence of MPCEs at any dose or harvest time in either sex. No significant difference was observed in the ratio of PCEs: NCEs for males, but the ratio for female was significantly decreased in the 48-hour group at 200 mg/kg. Therefore, it was considered that the effect in the females was caused by the test substance. Significant ($p<0.01$) increases in MPCE were induced by the positive control (60 mg/kg CP) in both sexes.

Table 1a: Summarized results of the micronucleus assay in male rats treated with S-1563 [a]

	Vehicle control group		Positive control group (CP) [b]	S-1563			
				150 mg/kg	300 mg/kg	600 mg kg	
Number of cells evaluated (PCEs /animal) [b]	2000		2000	2000	2000	2000	
Sampling time (hours)	24	48	24	24	24	24	48
Number (% mean) of MPCEs [b]	0.18	0.19	2.38**	0.18	0.14	0.20	0.15
Ratio of (% mean) PCE:(PCE + NCE) [b,c]	59.6	48.0	26.3**	56.4	62.2	55.2	56.7

[a] Data were extracted from the study report, Table 6, p. 26 (MRID 49020032)
[b] Abbreviations: PCE = polychromatic erythrocytes; MPCE = micronucleated polychromatic erythrocytes; NCE= normochromatic erythrocytes; CP = cyclophosphamide
[c]: One thousand erythrocytes were analyzed from each animal.
** $p<0.01$

Table 1b: Summarized results of the micronucleus assay in female rats treated with S-1563 [a]

	Vehicle control group		Positive control group (CP) [b]	S-1563			
				50 mg/kg	100 mg/kg	200 mg kg	
Number of cells evaluated (PCEs /animal) [b]	2000		2000	2000	2000	2000	
Sampling time (hours)	24	48	24	24	24	24	48
Number (% mean) of MPCEs [b]	0.08	0.14	1.14	0.12	0.09	0.15	0.14
Ratio of (% mean) PCE: (PCE + NCE) [b,c]	45.4	54.4	21.2**	49.4	46.7	54.3	40.0*

[a] Data were extracted from the study report, Table 7, p. 27 (MRID 49020032)
[b] Abbreviations: PCE = polychromatic erythrocytes; MPCE = micronucleated polychromatic erythrocytes; NCE= normochromatic erythrocytes; CP = cyclophosphamide
[c]: One thousand erythrocytes were analyzed from each animal.
** $p<0.01$

III. CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The study author concluded that S-1563 did not induce micronuclei in rat bone marrow cells under the conditions tested. It is therefore concluded that there is no evidence for clastogenic activity of S-1563 in this test system.

B. REVIEWERS' COMMENTS: The reviewers agree with the investigator's conclusions. S-1563 was tested up to high doses causing overt toxicity in the males (600 mg/kg) and females (200 mg/kg) and a cytotoxic response in the female bone marrow but failed to induce an aneugenic or clastogenic response. The sensitivity of the test system to detect a genotoxic response was demonstrated by the positive control. Based on these considerations, it was concluded that S-1563 was negative in this in vivo test system in a well conducted assay.

C. STUDY DEFICIENCIES: None